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13. ABSTRACT (Maximum 200 words)

Future advances in computing technology require exploration of next generation strategies. Approaches that use the ultra-parallel processing of bio-molecular procedures are one of the most intriguing strategies for new computing paradigms. These strategies provide for the solution of many algorithms in a linear number of steps compared to an exponential number of steps with traditional computing. Using DNA as the memory register in a fluid environment like water is a strategy called "aqueous computing." It has been used to achieve successful solution of simple algorithms based on a restriction enzyme writing procedure. While the proof of concept for this approach has been achieved, a number of challenges have been identified including the long time required. For this grant, another strategy for writing on DNA was investigated using sequence specific DNA methylation, and this strategy was used to solve a 4-variable SAT problem.

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(Continuation Sheet)

Final Report for ARO the award: 'DNA Based Fluid Computing using Methylation'
made to Binghamton University, Binghamton, NY
August 1, 2004 - April 30, 2005 Amount = $38,515
PI: Thomas J Head Co-PI: Susannah Gal

The PIs proposed to investigate the possibility of 'writing on' and 'reading from' DNA molecules using methylating enzymes for writing and restriction enzymes for reading. The concept requires that enzyme pairs be chosen each of which consists of one methylating enzyme and one restriction enzyme with the property that *the restriction enzyme will cut a DNA molecule **if and only if** its corresponding methylating enzyme has not been applied previously*. The possibility of using methylation as a technique in DNA computing was completely new and untested. The following eight pairs were chosen and were laboratory tested as an eight pair system for which the italicized requirement above holds. Moreover each methylase blocks only its own restriction enzyme.

Listing of methylase and restriction enzymes used for computation

Methylase	Seq. modified[1]	Restriction enzyme blocked
BamHI methylase	G-G-A-T-^{m}C-C	BamHI GGATCC
Dam methylase	G-^{m}A-T-C	DpnII GATC
ClaI methylase	A-T-C-G-^{m}A-T	ClaI ATCGAT
EcoRI methylase	G-A-^{m}A-T-T-C	EcoRI GAATTC
HaeIII methylase	G-G-^{m}C-C	NotI GGCGGCCGC
HhaI methylase	G-^{m}C-G-C	BssHII GCGCGC
HindIII methylase	^{m}A-A-G-C-T-T	HindIII AAGCTT
HpaII methylase	C-^{m}C-G-G	SmaI CCCGGG

1-Sequence after methylase modification with the ^{m}A or ^{m}C indicating a methylated adenine or cytosine residue, respectively.

With these eight choices made and tested, we began the test of the use of six of these pairs in an example computation. Previously we had carried through the solution of a three-variable four-clause instance of the Boolean satisfiability problem (SAT) by writing on & reading from circular DNA molecules. Our previous technique for writing was a three-step procedure consisting of Cutting, Extending, and re-Ligating (CEL) the DNA molecules. The point of our present proposal was to investigate the possibility of replacing the time consuming and error-prone CEL operation by methylation. To allow the most direct comparison of our newly proposed writing by methylation with our previous CEL-writing, we elected to carryout the same three-variable four-clause SAT. Two slightly different sources of the DNA molecules were used as the 'tablets' on which to write: the same circular plasmid used previously (pBluescript), and the much shorter linear segment obtained by copying by PCR the crucial portion of the plasmid that contains all the relevant enzyme sites. By the termination date of our grant we had only partial success with each of these computations. The solution expected for this SAT is: FTF, meaning the truth values of the propositional variables p,q,r for which all four clauses evaluate to T (True) are: p=F (False), q=T, r=F. Both of our computations agreed nicely in assigning p=F & r=F, but the result q=T was not unambiguously obtained. Our wet lab work produced the contradiction: q=T &. q'=T.. Reading was done through bands produces in gels by electrophoresis. In one of the cases the

band indicating q=T was visibly stronger than the band indicating q=F, but we cannot consider this to be fully satisfactory. It appears that either the methylase-restriction pair devoted to q did not work correctly or that there is an undesired interaction among the system of six enzymes chosen. In both cases, the restriction enzymes used for the q/q' site were NotI and BssHII which might indicate that these enzymes are not working sufficiently well for our purposes.

The Co-PI, Susannah Gal, will be continuing laboratory work in an effort to resolve the problem mentioned. Moreover she plans to investigate the use of fluorescently labeled DNA to further simplify the reading process in computations.

Summary of achievements under this grant at the date of its conclusion:
(1) We have chosen and tested methylation / restriction pairs that show promise for use in DNA computing. (2) Our work was presented in the poster session held at the 11th International Workshop on DNA Computing held in June 2005 at the University of Western Ontario where it was well received and invited for potential publication. (3) Even if the wrinkles mentioned above cannot be ironed out completely, we are confident of eventual publication of our laboratory results.